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THE METABOLISM OF CERTAIN RAPIDLY GROWING HUMAN TUBERCLE BACILLI IN A MODIFIED USCHINSKY MEDIUM

STUDIES IN ACID-FAST BACTERIA. III*

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In the previous articles the recession of ammonia was observed in old cultures of avirulent, rapidly growing cultures of tubercle bacilli. The cause of this recession was unknown, but probably it is not attributable to small amounts of lipoids or fats.

The present study was made to determine the incidence of this reaction in media of very simple composition. The experiments of Proskauer and Beck,[1] which have since been repeated by many observers,[2] have shown that the human tubercle bacillus can grow in media of relatively simple composition. These media would seem to offer advantages for the study of the recession of ammonia which we have observed. This advantage lies in the fact that it is possible to provide, as a source of nitrogen, a chemical compound the composition of which is definitely known. For the purpose of this investigation, a medium was made consisting of 4 gm. of asparagin, 2 gm. of di-sodium hydrogen phosphate, and 5 gm. of NaCl to the liter of distilled water, as a basis. This medium was divided into three parts: to one of which was added 1 percent of dextrose; to a second, 1 percent of mannite, and to a third, 3 percent of glycerin, as additional sources of carbon. They were then sterilized under parallel conditions, in 100 c.c. amounts. All of the utensils, which were used in the preparation of these media, were thoroughly freed from all organic matter. It will be seen that the source of nitrogen in this medium is asparagin. The source of carbon is selective, the organisms having a choice of the carbon from the asparagin or from dextrose, mannite, or glycerin, respectively. An attempt was made to grow the organisms in the asparagin solution without any additional source of carbon, but this was unsuccessful.

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[1] Ztschr. f. Hyg. u. Infectionskrankh., 1894, 18, p. 128.

[2] Frankel, Hyg. Rundschau, 1894, p. 769; Frouin, Compt. rend. Soc. de biol., 1910, 68, p. 915; Lowenstein, Centralbl. f. Bakteriol., Abt. I, Orig., 1913, 68, p. 591; Wherry, Ibid., 70, p. 115; and Jour. Infect. Dis., 1913, 13, p. 144.

TABLE 1

METABOLISM OF TUBERCLE BACILLI IN A MODIFIED USCHINSKY MEDIUM

Bacillus tuberculosis	Days	Dextrose Broth					Mannite Broth					Glycerin Broth				
		Alizarin	Neutral Red	Phenolphthalein	NH_3 mgs. Increase per 100 c.c. Broth	NH_3 / Total N_2 Percent	Alizarin	Neutral Red	Phenolphthalein	NH_3 mgs. Increase per 100 c.c. Broth	NH_3 / Total N_2 Percent	Alizarin	Neutral Red	Phenolphthalein	NH_3 mgs. Increase per 100 c.c. Broth	NH_3 / Total N_2 Percent
WI	7	0.00	+0.20	—0.10	4.9	6.60	—0.30	+0.20	0.00	11.2	12.5	—0.20	+0.10	+0.20	4.2	6.52
	14	—0.10	+0.20	+0.20	4.9	6.60	—0.30	+0.10	+0.20	25.9	39.4	0.00	+0.10	+0.10	4.9	7.61
	21	—0.20	+0.10	+0.40	23.8	32.1	—0.30	—0.10	+0.20	30.8	46.8	—0.10	—0.10	+0.30	9.8	15.2
	28	—0.20	0.00	+0.30	36.4	49.1	—0.50	—0.20	+0.20	39.9	60.3	—0.20	—0.20	+0.30	28.0	43.4
	35	—0.20	+0.40	+0.20	30.8	41.5	—0.30	+0.10	+0.30	30.8	46.8	—0.20	0.00	0.00	30.8	47.8
	42	—0.10	+0.10	+0.10	34.3	46.3	—0.60	—0.10	+0.10	31.5	48.0	—0.20	—0.10	+0.10	33.6	52.1
WII	7	0.00	+0.20	+0.10	4.2	4.55	—0.10	+0.20	+0.10	7.7	8.6	—0.10	+0.20	+0.30	4.2	6.52
	14	—0.10	+0.10	+0.10	6.3	8.49	—0.30	0.00	+0.30	23.1	41.8	—0.10	0.00	+0.20	6.3	9.78
	21	—0.20	0.00	0.00	14.0	18.9	—0.30	—0.20	+0.20	31.5	48.0	—0.10	—0.10	+0.20	14.0	21.8
	28	—0.20	+0.10	+0.20	36.4	49.1	—0.60	—0.20	+0.10	37.8	57.5	—0.20	—0.10	+0.20	27.3	42.4
	35	—0.20	+0.20	+0.30	31.5	42.4	—0.40	+0.10	0.00	30.8	46.8	—0.20	+0.10	+0.10	32.2	50.0
	42	—0.20	+0.10	+0.30	33.6	45.3	—0.40	0.00	+0.10	33.6	51.1	—0.20	0.00	+0.10	35.0	54.4
597I	7	0.00	+0.20	+0.10	3.5	4.87	—0.20	+0.20	+0.10	7.0	7.81	—0.10	+0.10	0.00	2.8	4.35
	14	0.00	+0.30	—0.10	4.9	6.60	—0.40	+0.10	0.00	21.0	31.9	0.00	+0.10	+0.20	2.1	3.26
	21	—0.10	0.00	+0.50	18.2	24.5	—0.50	—0.20	+0.10	30.1	45.8	—0.10	0.00	+0.20	23.8	37.6
	28	—0.10	+0.10	+0.50	32.9	44.3	—0.30	—0.10	+0.10	39.9	60.3	—0.20	—0.10	+0.20	25.2	39.3
	35	0.00	+0.30	+0.50	30.1	40.5	—0.30	+0.10	+0.10	32.2	49.0	—0.10	0.00	+0.20	28.0	43.4
	42	—0.10	0.00	+0.20	32.2	43.4	—0.30	—0.10	0.00	31.5	48.0	—0.10	0.00	+0.40	31.5	44.7
597II	7	0.00	+0.10	+0.30	4.2	4.55	—0.20	+0.20	+0.20	7.0	7.81	0.00	0.00	+0.10	2.1	3.26
	14	0.00	+0.30	0.00	5.6	7.54	—0.40	—0.10	+0.10	21.0	31.9	0.00	0.00	+0.30	2.1	3.26
	21	—0.10	0.00	+0.30	17.5	23.6	—0.30	—0.20	+0.10	30.1	45.8	—0.05	0.00	+0.30	10.5	16.3
	28	—0.10	+0.10	+0.30	31.5	42.4	—0.40	—0.20	+0.10	39.9	60.3	—0.05	—0.20	+0.30	25.2	39.3
	35	—0.20	+0.20	+0.30	31.5	42.4	—0.10	+0.20	+0.10	31.5	48.0	—0.05	0.00	+0.10	23.8	37.6
	42	—0.10	0.00	+0.30	31.5	42.4	—0.30	—0.10	+0.20	31.5	48.0	—0.10	—0.10	+0.30	32.2	50.0

The determinations were made in duplicate at weekly intervals for six weeks, and, with the exception of glycerin, the same recession of ammonia appears, as was noted in the previous experiments. The glycerin does not show this recession. The extent of the recession is somewhat less marked than in peptone-containing media, but the growth, it should be noted parenthetically, was much less luxuriant. Nevertheless, the changes induced in these media, both in reaction and in the decomposition of the nitrogenous constituents, were of sufficient magnitude to warrant the conclusion that the bacteria grew with moderate luxuriance. It was observed previously (Study II) in a medium containing peptone, which had been thoroughly extracted with organic solvents, that the reaction became progressively alkaline in glycerin. In the asparagin medium containing glycerin, the reaction was slightly, but consistently, acid to phenolphthalein. This was also the case in the dextrose and mannite media. The reaction with alizarin as an indicator, on the contrary, was uniformly alkaline. The amount of acid produced, however, was very slight, amounting to from 0.3 to 0.5 c.c. of normal acid per 100 c.c. of medium. Inasmuch as these organisms undoubtedly derive their nitrogen from the nitrogen of the asparagin, it might be assumed that the acid formed was due to the removal of the basic group of the asparagin. There is a certain amount of evidence in favor of this supposition, for the organism in question would not develop in this asparagin solution without an additional source of carbon, either mannite, gelatin, or dextrose, in the experiments cited. At the same time, the breakdown of asparagin, as measured by the increase in ammonia, was so great that it would be unjustifiable to assert that these substances exert a sparing action for the nitrogen. The extent of the breakdown of asparagin was, roughly, the same in each of these media. Altho the maximum degree of nitrogen metabolism was apparently not reached even at the end of six weeks in the glycerin medium, at the end of four weeks, generally speaking, the maximum growth was reached in the other media.

It is a noteworthy fact that, even in these very simple media, the tubercle bacilli, particularly during the last three weeks of growth, were completely acid-fast. During the initial stages of growth, that is, during the first and second weeks, there was a fair proportion of non-acid-fast rods. This is in harmony with the observations made in previous experiments in which more complicated media were used. Morphologically, the organisms were indistinguishable from those grown on the customary glycerin media, and their staining reactions, when judged by the rather crude methods available at the present time, particularly acid-fastness, were the same as those of tubercle bacilli derived from tuberculous lesions or cultures of tubercle bacilli of maximum virulence on ordinary media. In other words, the tubercle bacillus is able to build up the nitrogenous portion of its substance from asparagin, a relatively simple amino acid derivative, and its acid-fastness, be it a wax or a fat, from substances no more complex than dextrose, mannite, or glycerin, and perhaps some carbon from asparagin.

One of the noteworthy changes produced in the media by the growth of the organisms in the asparagin medium was the development of a mucinous-like substance, which was apparent even at the end of the first week. By the end of the second week it had apparently reached its maximum, altho it persisted throughout the course of the experiment. It was possible to draw out the medium in long and viscid strings by touching it with a platinum needle. Altho the medium underlying the pellicle of the tubercle bacillus exhibited this mucinous change most strongly, the organisms themselves were also somewhat mucinous in character. This viscosity was most marked in mannite, considerable in dextrose, and relatively slight in glycerin.

The relative luxuriance of growth of tubercle bacilli in this very simple medium has somewhat more than academic interest, for it has been shown by various authors that the tubercle bacillus can produce tuberculin from these compounds. Proskauer and Beck[1] showed that their cultures of tubercle bacilli would produce tuberculin in their asparagin medium, and this observation has been confirmed a number of times since. It would appear to be desirable to prepare tuberculin from a simple medium of this type, if possible, because the amount of extraneous substance is very little, consequently the reactions induced by it should be more clean-cut and less liable to misinterpretation, due to the elimination of the irritant action of peptone and other products of protein disintegration which contaminate tuberculin prepared in the usual manner.

The composition of commercial tuberculin varies greatly (White and Hollender[3]), and it is conceivable that tuberculin developed in a medium of known composition could be standardized much more accurately when it is relatively free from extraneous products than

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3. Trans. National Assn. for Study and Prev. Tuberc., 1913, 9, p. 326.

when it is mixed with substances of unknown composition and of themselves irritant.

After this work was completed, an article by Möllers[4] appeared, which would seem to indicate that, in an asparagin medium containing glycerin, there is a very considerable antigen content. It is interesting to note that the maximum antigen content coincides, in time, with the greatest weight of the pellicle formed on the surface of the medium, as shown by Möllers[4] and Lockemann.[5] After reaching a maximum, both the weight of the pellicle and the antigen content, according to their observations, decrease materially in amount. Möllers concludes that the course of the weight curve of the tubercle bacillus in fluid media shows a great similarity to the curve of the antigen content of the corresponding culture fluid. It was an interesting and significant fact that the greatest weight of the pellicle of the tubercle bacillus reached a maximum and then decreased, suggesting a relationship with the formation of ammonia, which also reaches a maximum and is then followed by a recession of the ammonia content. It is conceivable that the three phenomena, the decrease in weight of the tubercle bacillus pellicle, the decrease in antigen content, and the decrease in vegetative activity, as shown by the ammonia curve, are parallel phenomena, and the cause of the decrease in each instance is closely associated with the recession of this vegetative activity.

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[4] Möllers. Veröffentlichgungen der Robert Koch-Stiftung zur Bekämpfung der Tuberkulose, 1914, 10, p. 56.

[5] Lockemann, Ibid., p. 21.